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C6F
(71) Applicant
F. Hoffmann-La Roche and Co AG (Switzerland), 124—184 Grenzacherstrasse, Basle, Switzerland
(72) Inventors
Theophil Staehelin, Christian Stahl, Vincenzo Miggiano
(74) Agent and/or Address for Service
Carpmaels and Ransford, 43 Bloomsbury Square, London WC1A 2RA

(54) Monoclonal antibodies to human leucocyte interferons

(57) There is described a collection of monoclonal antibodies the members of which being directed against human leucocyte interferon (HLI) which is natural or obtained by recombinant DNA technology. The monoclonal antibodies are preferably secreted by different hybridomas. It has been shown that individual monoclonal antibodies are not mutually inhibiting, i.e. bind together to HLI while others are mutually inhibiting, i.e. do not bind together to HLI. It has been shown furthermore that the collection consists of groups of antibodies, members of which recognize different epitopes (antigenic determinants) fo HLI. Each a member of the different groups of antibodies can be used for a solid phase-sandwich test for HLI. In addition an antibody of the collection can be used for the purification of HLI by affinity chromatography.

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SPECIFICATION

Antibodies to proteins

The present invention is concerned with antibodies against proteins, namely against human leucocyte interferon (HLI) which is natural or obtained by recombinant DNA technology.

HLI is possibly a valuable therapeutic for the treatment of neoplastic and viral illnesses. The evaluation of this substance and its possible development to a widely-used medicament is restricted by the difficulty of purifying and of characterizing the active substance in the requisite manner. A test which could be carried out in less than 24 hours would be a valuable aid for the desired purification and the requisite characterization of HLI.

In the scope of the present invention it has now been found that for the purification and determination of HLI in an excellent manner there is suitable a collection of monoclonal antibodies which are directed against leucocyte interferon which is natural or obtained by recombinant DNA technology.

The present invention is accordingly concerned with a collection of monoclonal antibodies, which is characterized in that its members are directed against human leucocyte interferon (HLI) which is natural or obtained by recombinant DNA technology. The word collection denotes in this connection the entirety of antibodies as well as the individual members.

According to a particular aspect of the present invention, the monoclonal antibodies are secreted by different hybridomas.

In the scope of the present invention it has been shown that individual monoclonal antibodies are not mutually inhibiting, i.e. bind together to HLI, while others are mutually inhibiting, i.e. do not simultaneously bind to HLI. Furthermore, it has been shown that the collection consists of groups of monoclonal antibodies, members of which recognize different epitopes (antigenic determinants) of HLI.

One characteristic of the collection of monoclonal antibodies in accordance with the present invention consists in that at least one antibody does not recognize HLI γ_2.

In the scope of the present invention it has further been shown that the previously mentioned collection consists of a group of antibodies, which differ from one another in their isotypes. 8 antibodies show IgG structure, whereas one shows IgM structure. Of the 8 antibodies with IgG structure, 7 have a γ_1 heavy chain and one has a γ_{2b} heavy chain.

A further feature of the collection of monoclonal antibodies in accordance with the present invention consists in that one antibody (IgM) does not neutralize the antiviral activity of human leucocyte interferon.

As already mentioned, the collection of these monoclonal antibodies inter alia is characterized in that it consists of groups of antibodies, members of which recognize different epitopes of HLI. A further aspect of the present invention is accordingly concerned with the use of in each case one member of this group of antibodies for a solid phase-sandwich test for HLI. In the case of the previously mentioned sandwich test, an antibody is provided with a suitable label. For this there comes into consideration any label which is suitable for this purpose, but a radioactive or an enzyme label is preferred.

A further aspect of this present invention is concerned with the use of an antibody from the above-mentioned collection for the purification of HLI. The purification of HLI by means of the monoclonal antibody is preferably effected by affinity chromatography. For the purification in a commercial scale the affinity should be high enough so that the interferon is practically quantitatively retained also with great volumes which pass the column with a not too low speed. With a single antibody this is not possible for all sub-classes of interferon. For this purpose a collection of monoclonal antibodies which individual members show a high affinity for particular sub-classes of interferon is especially suitable.

The preparation of the monoclonal antibodies is effected according to cell fusion technology known per se, in which the myeloma cells are fused with spleen cells of mice, which have been immunized with HLI. The hybridomas obtained secrete monoclonal antibodies against leucocyte interferon which is natural or obtained by recombinant DNA technology.

The carrying out of the solid phase-sandwich test for the determination of HLI can be effected according to methods known per se, but a substantial simplification can be met with, in contrast to the known solid phase-sandwich procedure, by incubating unlabelled and labelled antibodies from the beginning together and only once.

The purification of HLI by means of a monoclonal antibody can be effected according to methods known per se, whereby the affinity chromatography has been found to be particularly suitable. In this affinity chromatography Affi-Gel 10 (BioRad Laboratories, Richmond, California) is preferably used as the carrier.

It has surprisingly been shown that, according to the solid phase-sandwich procedure in accordance with the present invention, human leucocyte interferon is detectable up to a concentration of 2 U/ml in serum.

On the other hand, it has been shown that human leucocyte interferon obtained by recombinant DNA technology can be purified with a monoclonal antibody in accordance with the present invention in a simple manner such that it is usable for clinical trials.

The following Examples illustrate the invention.

EXAMPLE 1

Manufacture of the monoclonal antibodies

A) Interferon preparation

Partially purified human leucocyte interferon (IFL) was available in sufficient amount for the immunization of the mice. 5 IFL preparations are used:

a) IFL γ(a) main peak of the fraction in accordance with step 8 of Table 3 of DOS No. 29 47 134 with an approximate purity of 10—15% (estimated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis) and specific activity in the antiviral test;

b) IFL α; c) IFL β; d) IFL γ; and e) IFL δ.

The IFL α, β and γ fractions consist in each case of mixtures of the individual (α_1, α_2), (β_1, β_2, β_3) and (γ_1, γ_2, γ_3, γ_4, γ_5) species. These are shoulder fractions of the corresponding purified species, which are pooled from different preparations (see step 9 of Table 3 of the previously mentioned DOS). The IFL δ fraction is the shoulder of the δ-species. Besides the species α, β and γ on the Lichosorb-diol column (see step 8 of Table 3 of the previously mentioned DOS) there is occasionally seen a δ-fraction. This is purified as in step 9 in accordance with Table 3 of the previously mentioned DOS, whereby it is evident that the species δ shows no sub-species and is uniform. The purity of b), c) and d) is between 20 and 40% in accordance with specific biological activity and in accordance with sodium dodecyl sulphate-polyacrylamide gel electrophoresis. The purity of e) is at most 5%.

Determination of interferon activity

The interferon activity is determined by means of the "cytopathic-effect-inhibition" (CPE) test in accordance with U.S. Patent Specification No. 4 241 174 (Serial No. 963,256).

B) Immunization of the mice

3 eight weeks old Balb c/J female mice are firstly immunized with IFL γ(a) in Freund's complete adjuvant. Each mouse receives approximately 150 μg total protein containing 20—25 μg of interferon in 0.25 ml at 5 different positions (0.05 ml in each position): subcutaneously in the left and right iliac region and in the left and right auxiliary region as well as an intraperitoneal injection.

53 days later a second immunization is carried out as follows: interferon IFL γ(a) is separated by preparative sodium dodecyl sulphate-polyacrylamide (15%) gel electrophoresis in three 0.6 x 11 cm cylindrical gels. Approximately 300 μg total protein in 0.3 ml (dialyzed against assay buffer) containing approximately 40—50 μg of interferon are loaded per gel. [This purification is effected essentially according to the method of Laemmli, U.K. (1970) Nature 227, 680—685]. After the electrophoresis, the gels are sliced into 2 mm thick discs. These are immersed for 10 minutes in 0.5 ml of phosphate-buffered sodium chloride solution (0.01 M potassium phosphate buffer, pH 7.3; 0.14 M sodium chloride) containing 0.1% Triton X—100 in propylene test tubes. The buffer is investigated for interferon activity in serial dilution according to the CPE inhibition test. The discs 6 and 7 (numbered from the lower end of each gel) show the highest interferon activity (in each case approximately 25% of the activity of the total gel). In each case one disc with highest activity is finely sliced with a razor blade on a glass plate, transferred into a 1 ml syringe containing 0.2 ml of 0.15 M sodium chloride and injected into each mouse. The gel suspension is injected intraperitoneally, whereupon 0.2 ml of BCG (Bacillus Calmette-Guérin; Serum Institute Berne) are injected.

After 12 days, the serum of each mouse is investigated for interferon neutralization activity. The serum of mouse No. 3 shows a 50% neutralization of 10 units/ml of IFL γ(a) at a dilution of 1:72 000, but does not neutralize the same concentration of crude IFL (< 0.1% purity) even at a dilution of 1:100. The mice No. 1 and No. 2 show neutralization titres of less than 1:1000 against IFL γ(a).

70 days after the second immunization mouse No. 3 receives on three successive days an intraperitoneal booster injection with a mixture of IFL α, β and γ containing approximately 50 μg of interferon and 15 μl of normal mouse serum as the carrier protein (in 0.2 ml of 0.15 M sodium chloride). 48 hours after the last injection the mouse is killed and the spleen is removed for the preparation of the monoclonal antibodies.

C) Cell cultures and cell fusions

The following materials and media are used. Iscove's modification of Dulbecco's modified Eagle medium (IMDMEM) is obtainable from Gibco. It is made up freshly with sodium pyruvate (1 mM), glutamine (1.5 mM), 2-mercapto-ethanol (5×10^{-5}M), penicillin (100 units/ml) and streptomycin (100 μg/ml). Complete HAT medium consists of thus-completed IMDMEM as well as hypoxanthine (10^{-4}M), aminopterin (4×10^{-7}M), thymidine (1.5×10^{-5}M) and 15% foetal calf serum. A 50% (w/v) solution of polyethyleneglycol 4000 (PEG 4000, Merck) in IMDMEM is prepared.

The fusion with a non-producing azaguanine-resistant myeloma cell line is carried out, with small modifications, according to the method of Stähli et al. in J. Immunol. Methods *32*, 297—304. In this fusion 48×10^6 nucleated spleen cells are fused with 25×10^6 myeloma cells of the line FO (Fazekas

de St. Groth and Scheidegger in J. Immunol. Methods *35*, 1—25 (1980). The spleen cells and myeloma cells are washed in serum-free IMDMEM. They are then re-suspended in the same medium, mixed in the above-mentioned ratio for the fusion and sedimented in 40 ml of serum-free IMDMEM in a 50 ml conical polypropylene test tube at 200 x g for 15 minutes, followed by complete sucking off of the supernatant. To the cell sediment there are added dropwise with constant stirring 0.5 ml of 50% PEG 4000 in order to re-suspend and to disperse the cells. After approximately 90 seconds, 10 ml of serum-free IMDMEM are added dropwise at room temperature with constant stirring during 4—5 minutes. After a further 15 minutes without stirring, large cell clumps are separated by cautious pipetting with a 10 ml pipette. The fusion mixture is diluted to 250 ml with complete HAT medium and then placed in 240 "Costar cluster wells (1 ml/well)", which already contain 1 ml of complete HAT medium and 10^5 peritoneal mouse cells as the nutritive layer. The cultures are incubated in a 5% CO_2/95% air atmosphere at 95% humidity. The cultures are fed twice a week by replacing half of the medium 1 ml) with fresh HAT medium

D) Detection of interferon-specific hybridomas by a solid phase-antibody binding test (SABA)

This method is adapted from the principle described by Catt and Tregear [Science *158*, 1570—1572 (1967)] and is suitable for the detection of hybridoma antibodies (see also Stähli et al in J. Immunol. Methods *32*, 297—304 (1980) as well as Kennet, R. H. in Current Topics in Microbiology and Immunology, Vol. 81, p. 77—91 (1978). Interferon preparations of IFL α, β, γ and δ are individually diluted in polypropylene test tubes with phosphate-buffered sodium chloride solution (PBS) to an end concentration of approximately 0.2 to 0.5 μg interferon/ml (approximately 1—3 μg total protein per ml; except for IFL δ where approximately 10—15 μg of total protein are present per ml), in order to coat therewith polyvinyl chloride microtitre plates (Cooke Laboratory Products Division, Dynatech Laboratories, Inc.) as in the case of Stähli et al. loc. cit. 50 μl of the interferon solution is placed in each well and left for at least 4 hours at room temperature in a humid chamber or held at (sic) 4°C for several weeks. Before use the wells are filled wtih 3% bovine serum albumin (BSA) in PBS and left for 30 to 60 minutes in order to block all protein binding positions. The plates are then washed four times with PBS. 50 μl of supernatant of the hybridoma cultures are incubated in in each case two wells for at least 4 hours at room temperature. Control experiments (unspecific binding) are carried out on plates which are coated only with 3% BSA. After four-fold washing with PBS, there is added to each well 50 μl of sheep-anti-mouse Ig-antibody (purified by affinity chromatography and labelled with ^{125}I; 50 000 to 100 000 cpm per 20 to 30 ng of antibody in PBS containing 1% BSA) and it is incubated at room temperature for at least 4 hours. The plates are washed four times to five times with PBS and then divided into the individual wells, which are then tested for radio-activity in a gamma-scintillation spectrometer

E) Results

a) *Initial screening by antibody binding (SABA) against partially purified IFL fractions*

152 of the 240 cultures show growth of hybridomas. The supernatants are tested for interferon-specific antibodies in the SABA test, when the cultures are approximately 20—30% confluent (12—29 days after the fusion). Antibody binding is tested parallel with IFL α, IFL β, IFL γ and IFL δ. Of the 152 cultures, 13 supernatants show high antibody binding against all four partially purified interferon preparations. The medium of four further hybridomas shows lower binding activity (3—4 times greater than unspecific binding) against all four interferon preparations. These cultures are discarded. The media of all other hybridomas (135 growing cultures) yield unspecific bindings against all four IFL preparations. Of the 13 specific hybridomas, 9 are characterized representative for the present invention. Their designation is LI—1, LI—2, LI—3, LI—5, LI—6, LI—7, LI—8, LI—9 and LI—12.

As is evident from Figure 1, the antibody binding of the 9 specific hybridomas shows a different behaviour to the 4 partially purified interferon preparations. LI—1, LI—2, LI—3, LI—5, LI—6, LI—7 and LI—8 show an identical characterization with the following binding sequence: IFL α < IFL δ < IFL γ < IFL β. For LI—9 the binding order is IFL δ < IFL γ < IFL β. LI—12 is different from all others.

b) *Antibody binding (SABA) against highly purified leukocyte interferon*

Six purified (≥ 80% pure) interferon species (see step 9 of Table 3 of DOS 29 47 134) are immobilized by adsorption on polyvinyl chloride microtitre plates. In this case there are concerned IFL α_1, α_2, β_2, γ_2 and γ_3 as well as IFL—K of the cell line KG—1 [Koeffler, H.P. et al. Science *200*, 1153—1154 (1978)], which is purified in analogy to the steps 1 to 9 of Table 3 of the mentioned DOS. Since no clear separation into individual species was to be observed in the steps 8 and 9, the main fraction with the interferon activity was pooled. It accordingly represents a mixture of different species. The adsorption of interferon (50 μl/per depression) is carried out overnight with approximately 0.5 μg of interferon and 2 μg of BSA per ml of PBS followed by blocking off of the protein binding positions with 3% PBS as described previously.

The antibody binding with the culture medium of the 9 specific hybridomas against the 6 different, purified interferons is shown in Figure 2. There can again be differentiated various groups of antibodies with different characteristic binding behaviour. The group 1 embraces the antibodies LI—1 and LI—2, which have identical binding characteristics, but which do not recognize IFL γ_3. The group 2 embraces

the antibodies LI—3, LI—5, LI—6, LI—7 and LI—8 with a possible differentiation of two sub-groups. Sub-group 2a (LI—3, LI—5 and LI—6) shows overall higher binding (Figure 1 and Figure 2) than sub-group 2b (LI—7 and LI—8), whereby the latter sub-group binds relatively stronger to purified IFL α_1 and IFL—K than the first. According to the interferon binding behaviour of the remaining antibodies in accordance with Figure 2 and Figure 3, LI—9 has the most characteristics of sub-group 2b. LI—12 appears, in turn, to be unique.

Having regard to the fact of the high antibody binding to different purified interferons it is very unlikely that any of these antibodies was not directed against interferon, but against contaminants. This is indicated for most of these antibodies also by the capability of neutralizing the biological activity of interferon. LI—1, LI—2, LI—3, LI—5, LI—6, LI—7, LI—8 and LI—9 annul the inhibition of the viral CPE on MDBK cells induced by interferon. The interferon neutralization is tested with partially purified IFL γ(a) and/or purified IFL—K, IFL γ_1, IFL α_2 and crude leucocyte interferon. None of the antibodies was in a position to neutralize crude leucocyte interferon. LI—12 neutralized none of the tested interferons (see Table 1 for this).

2) *Isotypes of the heavy and light chains of the monoclonal interferon antibodies*

Monoclonal antibodies of hybridoma culture medium are firstly bound to interferon-coated microtitre plates as has been described previously for the SABA test. After washing, they are incubated with isotype-specific rabbit anti-mouse Ig antiserum (Nordic). For the detection there is used goat anti-rabbit IgG, which is labelled with horseradish-peroxidase. As the enzyme substrate there are used 2,2'-azino-di-(3-ethyl-benzothiazoline sulphate) and hydrogen peroxide. The results are compiled in Table 1, second column. Seven of the tested antibodies have the immunoglobulin chains γ_1/k, one has γ_{2b}/k and one has μ/k. All seven γ_1/k antibodies as well as also the γ_{2b}/k neutralize interferon, whereas the μ/k does not neutralize interferon, as is likewise evident from Table 1.

The ability of the antibodies according to the present invention to bind with high affinity to interferons obtained by recombinant DNA technology is evident from Table 6.

3) *Cloning and stabilization of the interferon-specific hybridomas*

For the preparation of stable hybridoma lines, all 9 specific hybridomas are cloned by limiting dilution in microtitre plates with mouse-peritoneal cells as the nutritive layer [Hengartner H. et al. in Current Topics in Microbiology and Immunology, Vol. 81, 92—99 (1978)]. The cloning was begun at the point in time of the transfer of the original cultures into flasks or shortly thereafter. The clones were tested against IFL β with the SABA test. The results of this first cloning of the 9 hybridomas is shown in Table 2.

From each hybridoma there are intensively cultivated two to four strongly positive clones and not only injected i.p. into mice for ascites production but also frozen. In most cases the selected clones of a hybridoma are pooled for simplification and reduction of work. From all 9 hybridomas there is obtained ascites liquor for the production of antibodies on a large scale. Ascites cells are likewise transferred into cultures and frozen.

4) *Grouping of the antibodies according to their interactions with HLI*

The main group of the seven hybridomas, which produce γ_1/k immunoglobulin, can be divided into two groups on the basis of their antibody interaction with purified interferons. The first group (LI—1 and LI—2) does not recognize purified IFL γ_2, whereas the second group (LI—3 and LI—5 to LI—8) recognizes IFL γ_2. The relative, quantitative binding of the monoclonal antibodies to six different purified interferons (see Figure 2) and the difference between neutralizing and non-neutralizing antibodies yields information for the differentiation of different epitopes (antigenic determinants) of HLI and for the demonstration of structural differences between different purified interferons. From the binding behaviour of the antibodies (see Figure 2) it is clear that LI—1 and LI—2 recognize a different structure than all other antibodies and that IFL γ_2 is structurally different from all other tested interferons, since it does not exhibit the epitope recognized by LI—1 and LI—2. LI—12, a non-neutralizing antibody, recognizes with approximately the same, although perhaps lower affinity, a less variable determinant which is present in all tested interferons. The results in respect of the antibody binding and of the interferon neutralization indicate that at least three different epitopes of HLI can be recognized and defined by the collection of the monoclonal antibodies.

EXAMPLE 2

Determination of interferon

1) Selection of a suitable pair of monoclonal anti-bodies

For the differentiation between competitive binding of two antibodies to the same epitope and additive binding to various epitopes, the binding of the antibodies is tested along and in pairs and in all possible combinations to immobilized interferon. Supernatants of confluent hybridoma cultures are used without further purification as the antibody source. Partially purified leucocyte interferon (20—30% pure IFL β) is added in defined amounts (0.1 to 0.2 μg interferon/ml) to polyvinyl chloride microtitre plates as is described in Example 1 for the SABA. The blocking off of all protein binding

positions with 3% BSA follows. 50 µl of antibody solution consisting of in each case 25 µl of two culture supernatants or 2 x 25 µl of the same antibody are incubated at room temperature for 7 hours. The plates are washed with PBS (0.01 M potassium phosphate, pH 7.3; 0.14 M sodium chloride) and incubated for 5 hours with an excess of ^{125}I labelled sheep-anti-mouse-Ig immunoglobulin in a volume of 50 µl per well in order to measure the amount of monoclonal antibodies which are bound to interferon (Table 3). The binding of two antibodies is considered to be additive when the binding of these anti-bodies exceeds that with 50 µl of the higher binding individual antibody. On the basis of this criterion it was found that LI—1 binds additively with almost all other antibodies and that LI—9 gives the best additive binding with LI—1.

LI—1 and LI—9 from ascites liquor are purified according to standard methods, i.e. by ammonium sulphate precipitation (50% saturation) and DEAE-cellulose chromatography, up to at least 90%, as assessed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. 20 µg of each antibody are labelled with ^{125}I according to the chloramine-T method to a specific activity of approximately 20 µCi/µg. Free iodine is separated from labelled antibody by gel filtration on Sephadex G—25 in the presence of PBS and 1% BSA.

Unlabelled antibodies LI—1 and LI—9 to approximately 20 µg per ml of PBS are adsorbed overnight on PVC microtitre plates (Cooke Laboratory, Products Division, Dynatech Laboratories, Inc.) (100 µl/depresssion). During 30 to 60 minutes all protein binding positions are blocked with 3% BSA in PBS. Purified leucocyte interferon of the KG—1 myeloid cell line at a concentration of 10 units/100 µl of PBS and 1% BSA are incubated together with one of the ^{125}I labelled antibodies (1.3 x 10^5 cpm) in antibody-coated wells. Control wells contain labelled antibody, but no interferon. After incubation for 3 hours at room temperature, the liquor is removed and the wells of the plates are washed four times with PBS and then tested for radioactivity in a gamma-scintillation spectrometer. The results are shown in Table 4. The combination of LI—1 with LI—9 gives interferon-dependent sandwich-like antibody binding. The better combination is immobilized LI—9 with labelled LI—1 in solution. For this reason the further experiments have been carried out with immobilized LI—9 and labelled LI—1.

a) *One-step Incubation procedure*

Each well of a PVC microtitre plate is coated with 100 µl of LI—9 (15 µg/ml in PBS) at room temperature overnight or at 4°C for days or weeks in a humid chamber. Before use the solution of LI—9 is removed and the wells are filled with 3% BSA for 30 to 60 minutes and then washed four times with PBS. Approximately 140 000 cpm of ^{125}I labelled LI—1 (approximately 4 ng, specific activity approximately 20 µCi/µg) in 0.1 ml of PBS containing 1% BSA and 100 to 150 µg of human IgG per ml are added to each well. Correspondingly diluted interferon solutions (1—20 µl) are added to the well and mixed by brief shaking. After 2 to 3 hour incubation at room temperature, the liquor is poured on to absorption paper. The plate is washed four times to five times with PBS and the individual wells are tested for radioactivity in a gamma-scintillation spectrometer. In the case of high interferon concentrations (> ca. 5000—6000 U/ml) a partial inhibition occurs. The results obtained are reproduced in Figure 3 and Table 5.

b) *Two-step incubation procedure*

LI—9 coated and BSA-saturated PVC microtitre plates are prepared as described previously. 50 µl per well of an interferon solution in PBS containing 1% BSA are incubated at room temperature for 30 minutes, whereupon the plates are washed four times with PBS. Thereupon, 50 µl of ^{125}I LI—1 (approximately 150 000 cpm/4 ng) PBS containing 1% BSA and 150 µg of human IgG per ml are added to each well, whereupon the plates are held at room temperature for 2 hours and then washed four times with PBS and tested for radioactivity as mentioned above. The results obtained are reproduced in Figure 4. In contrast to the one-step procedure no partial inhibition at high interferon concentration is seen.

c) *Determination of antiviral inactivated interferon*

Heat Inactivation of Interferon

A solution of Interferon A (Goeddel et al. Nature Vol. 290, March 1981) in Eagle's minimal essential medium containing 10% fetal bovine serum was diluted 1:480 in phosphate-buffered saline (0.14 M NaCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 3 mM KCl, 0.9 mM $CaCl_2$, 0.5 mM $MgCl_2$) containing 1 mg/ml of bovine serum albumin. The diluted solution contained Interferon A at a concentration of 5 ng/ml. After heating at 65°C for the times noted, samples of 0.14 ml were placed in an ice bath until assayed. Both antiviral and immunological assays were performed on the same day. From each 0.14 ml sample, 0.10 ml was used for the radio-immunoassay and 10 µl for antiviral assay in duplicate. The radio immuno assay is performed according to the two-step incubation procedure mentioned under b). The determination of the antiviral activity is performed according to the CPE-inhibition Test. The results are given in figure 6. From this example one can gather that the antibody pair LI—1/LI—9 can distinguish between antiviral active and antiviral inactive interferon. From figure 6 there is furthermore evident that the antiviral and the immune activity decrease simultaneously.

d) *Highly sensitive interferon determination in human plasma or serum (radioimmune procedure)*

A single incubation of 0.1 ml of 40% human plasma or serum (neutral pH) containing approximately 250 000 cpm (1.5 ng) of ^{125}I LI—1 (specific activity 100 to 110 μCi/μg LI—1) is carried out at room temperature for 14 to 16 hours in PVC microtitre plates, which are coated with LI—9 as described previously. After four-fold washing with PBS, the wells are numbered as mentioned previously. One experiment is carried out with interferon, which is added to citrate plasma of a healthy volunteer. Each 0.1 ml of PBS test mixture contains 40 μl of plasma, 1.3 mg of ^{125}I LI—1 (256 000 cpm) and different amounts of crude human leucocyte interferon (approximately 0.1% pure) from chronic myologous leukaemia cells. The results are re-produced in Figure 5, where the amount of interferon is plotted as the abscissa. The incubation medium in accordance with step 1 of Table 3 of DOS No. 29 47 134 is used as the crude leucocyte interferon.

EXAMPLE 3

Highly sensitive determination of interferon in serum of patients (enzyme immune procedure)

Into the corresponding number of test tubes (10 x 75 mm) there are in each case pipetted 0.05 ml of test solution (0.1 mol/l of sodium phosphate, pH 6.5 with 10 g/l of BSA and 0.5 μg/ml of monoclonal LI—1 anti-interferon-peroxidase conjugate in interferon-free human serum) 0.2 ml of the patient's plasma to be analyzed or of the interferon standard (0 U/ml, 12.5 U/ml, 25 U/ml and 50 U/ml of interferon) and of the interferon control serum (e.g. 30 U/ml of interferon) is admixed, in each case there is added a polystyrene bead (Ø = 6.5 mm) coated with monoclonal LI—9 anti-interferon and incubated at room temperature (18—20°C) for 16 hours. Subsequently, the polystyrene bead are washed three times with in each case 2 to 5 ml of distilled water, transferred into in each case 0.5 ml of substrate buffer for the determination of the activity of the peroxidase (0.1 mol/l of potassium citrate buffer of pH 5.0 with 6 mmol/l of H_2O_2 and 20 mmol/l of o-phenylenediamine) and incubated at room temperature (18—26°C) for 30 minutes. In order to stop the peroxidative activity as well as to intensify the light absorption, 0.5 ml of 4 N HCl are admixed and within 30 minutes the extinction is measured photometrically at the wavelength 492 nm. In the Table there are presented the values of an interferon determination and compared with the values which have been obtained with the interferon standard. Crude leucocyte interferon (incubation medium in accordance with step 1 of Table 3 of DOS 29 47 134) is used as the interferon standard.

TABLE

Sample material	$\Delta E_{492\ nm/RT/30\ min.}$
Interferon standard	
0 U/ml Interferon	0.065/0.070
12.5 U/ml ,,	0.245/0.235
25 U/ml ,,	0.425/0.430
50 U/ml ,,	0.775/0.790
Control serum (30 U/ml interferon)	0.505/0.490
Patient's plasma	
No 8327	0 U/ml Interferon
No 8328	0 U/ml Interferon
No 8336	9 U/ml Interferon
No 8338	0 U/ml Interferon
No 8339	5 U/ml Interferon
No 8363	0 U/ml Interferon
No 8341	2 U/ml Interferon
No 8344	16 U/ml Interferon

EXAMPLE 4

Purification of leucocyte interferon obtained by recombinant DNA technology (Interferon A, c.f. Goeddel et al. Nature, vol. 20, March 1981).

a) *Pre-purification of the human leucocyte interferon obtained by recombinant DNA technology*

All steps are carried out 4°C. Frozen bacteria cells (1 kg) are broken up by pressure disintigration. The extract is worked up according to the standard method, i.e. by polyethyleneimine (Polymin P) and ammonium sulphate precipitation, followed by a dialysis of the 65% ammonium sulphate precipitate against 25 mM Tris HCl, pH 7.8; 0.01% thiodiglycol; 0.1% Triton X—100; 10 μM phenyl fluoride. A centrifugation follows for the removal of insoluble constituents.

b) *Preparations of the immunoadsorbent*

In analogy to Example 2, purified antibody solution containing LI—8 is dialyzed at room temperature against 0.2 M sodium carbonate/0.3 sodium chloride buffer. The dialyzed solution is centrifuged at 20 000 x g for 15 minutes in order to remove insoluble material. The protein concentration is adjusted to about 25 mg/ml with the above-mentioned buffer. Affi-gel 10 (BioRad Laboratories, Richmond, California) is washed three times with ice-cold isopropanol on a sintered glass filter and then washed three times with ice-cold distilled water. The gel is transferred into plastic test tubes and sedimented by brief centrifugation. The supernatant is sucked off. The gel is mixed with an equal volume of purified antibody solution and rotated head over foot at 4°C for 5 hours. After the reaction, the gel is centrifuged and then washed twice with buffer (0.1 M $NaHCO_3$/0.15 M NaCl) in order to remove unbound antibody. Protein determination of the combined wash-water shows that about 24 mg of antibody were bound per ml of gel.

c) *Affinity-chromatographical purification of the pre-purified leucocyte interferon obtained by recombinant DNA technology*

The solution obtained in step a) (700 ml containing 37 g of protein) is placed at 50 ml/h on the immuno-absorbent column (2.5 x 3.5 cm); 17 ml bed volume containing about 400 mg of purified monoclonal antibody LI—8) equilibrated with PBS. The column is washed with 20 column volumes of buffer (0.5 M NaCl; 0.02 M Tris. HCl, pH 7.5; 0.2% Triton X—100), then the column is washed with 5 column volumes of 0.15 M NaCl, 0.1% Triton X—100 and then eluted with 0.2 M acetic acid, 0.15 M NaCl, 0.1% Triton X—100 (pH 2.5). The interferon activity is eluted in about 30 ml in an average concentration of approximately 1 mg protein/ml. This solution is adjusted to pH 4.5 with 1 M Tris base and diluted four-fold with water. The sample is loaded on to a carboxymethylcellulose (CM 52 Whatman) column (1.3 x 3 cm), which is equilibrated with 0.1 M ammonium acetate (pH 5.0). The column is washed with 20 ml of 0.1 M ammonium acetate (pH 5.0) and the interferon is eluted with 20 ml of 0.5 M ammonium acetate (pH 5.0). The result of the purification is reproduced in Table 7.

Interferon A can be purified in a similar manner and with a comparable yield with the antibodies LI—3, LI—5, LI—6, LI—7 and LI—9.

TABLE 1

Epitope	Monoclonal antibody	Isotypes	Chains	Neutralization
I	LI–1	IgG	γ_1/k	Yes
I	LI–2	IgG	γ_1/k	Yes
II	LI–3	IgG	γ_1/k	Yes
II	LI–5	IgG	γ_1/k	Yes
II	LI–6	IgG	γ_1/k	Yes
II	LI–7	IgG	γ_1/k	Yes
II	LI–8	IgG	γ_1/k	Yes
II	LI–9	IgG	γ_{2b}/k	Yes
III	LI–12	IgM	μ/k	No

TABLE 2

Culture	Cells per 96 "wells"	Tested clones or "wells"	Number positive	Number negative
LI–1	100	6	6	0
LI–2	100	11	6	5
LI–3	100	7	7	0
LI–5	100	13	13	0
LI–6	100	12	12	0
LI–7	100	10	8	2
LI–8	100	9	8	1
LI–9	100	10	8	2
LI–12	100	4	0	4
LI–12	300	48	6	42

TABLE 3

	LI–1	LI–2	LI–3	LI–5	LI–6	LI–7	LI–8	LI–9	LI–12
LI–1	15800	14980	17820	17730	15950	17670	17970	19550	18260
LI–2		11880	15120	16750	14570	16440	16150	16250	15460
LI–3			11600	13440	10320	11900	13270	9710	15490
LI–5				13640	11690	13230	14720	11820	16710
LI–6					7300	10290	10860	6540	12240
LI–7						12090	13480	8630	14610
LI–8							14700	10270	17680
LI–9								4900	10870
LI–12									9940

TABLE 4

Antibody combination		^{125}I labelled antibody binding (cpm)		
Solid phase antibody	^{125}I labelled antibody	with interferon (10 U)	without interferon	specific binding
LI–1	LI–9	922	440	482
LI–9	LI–1	2465	251	2208

TABLE 5

Interferon species	Interferon units per determination	CPM[^{125}I]LI–1 binding
α_1	100	2,202
α_2	100	1,633
β_1	100	822
β_2	100	8,135
β_3	100	70
γ_1	100	7,036
γ_2	100	4,804
γ_3	100	101
γ_4	100	198
γ_5	100	121
δ	100	5,498

TABLE 6

	Antibody						
Interferon	LI–1	LI–3	LI–5	LI–6	LI–7	LI–8	LI–9
A	+	+	+	+	+	+	+
B	+	–	–	–	–	–	–
D	–	+	+	+	+	+	+
F	–	–	–	–	±	–	–

Legend:

The interferons designated as A—F are those obtained by the DNA recombinant technology and described in the publication of David V. Goeddel et al. in Nature Vol. 290, March 1981. A + designates that a given interferon was completely absorbed, a ∓ partially absorbed and a — not absorbed by the columns loaded with the respective antibody.

TABLE 7

Step	Volume (ml)	Total Protein (mg)	Total activity (units)	Specific activity (units/mg)	Purification factor	Yield (%)
Ammonium sulphate precipitation	700	37,100	7.4×10^9	2×10^5	1.0	100
Antibody column pool	30	30	7×10^9	2.3×10^8	1150	95
CM–52	20	20	6×10^9	3×10^8	1500	81

The specific activity was determined according to the CPE inhibition procedure mentioned earlier. In accordance with sodium dodecyl sulphate-polyacrylamide gel electrophoresis the interferon after the immunoadsorption step is over 90% pure. According to the CM–52 column it appears to be practically homogeneous.

CLAIMS

1. Collection of monoclonal antibodies, characterized in that its members are directed against human leucocyte interferon (HLI) which is natural or obtained by recombinant DNA technology.

2. Collection according to claim 1, characterized in that the monoclonal antibodies are secreted by different hybridomas.

3. Collection according to claim 1, characterized in that it consists of groups of antibodies, which differ from one another in their isotypes.

4. Collection according to claim 2, characterized in that 8 antibodies are of IgG isotype and one antibody is of IgM isotype.

5. Collection according to claim 3, characterized in that of the 8 antibodies with IgG type 7 have a γ_1 heavy chain and one has a γ_{2b} heavy chain.

6. Collection according to claim 1, characterized in that individual monoclonal antibodies are not mutually inhibiting, i.e. bind together to HLI, while others are mutually inhibiting, i.e. do not bind together to HLI.

7. Collection according to claim 6, characterized in that it consists of groups of antibodies, members of which recognize different epitopes (antigenic determinants) of HLI.

8. Collection according to claim 1, characterized in that at least one antibody from the collection does not recognize HLI γ_3.

9. Collection according to claim 2, characterized in that one antibody (IgM) does not neutralize the antiviral activity of HLI.

10. Use of in each case one member from different groups of antibodies in accordance with claim 7 for a solid phase-sandwich test for HLI.

11. Use according to claim 10, characterized in that the non-immobilized antibody is labelled with radio-activity or an enzyme.

12. Use of an antibody in accordance with the collection of claim 1 for the purification of HLI which is natural or obtained by recombinant DNA technology.

13. A process for the purification of natural HLI or HLI obtained by recombinant DNA technology, characterized in that a HLI containing raw material is purified by affinity chromatography using a monoclonal antibody of the collection according to any one of claims 1—9.

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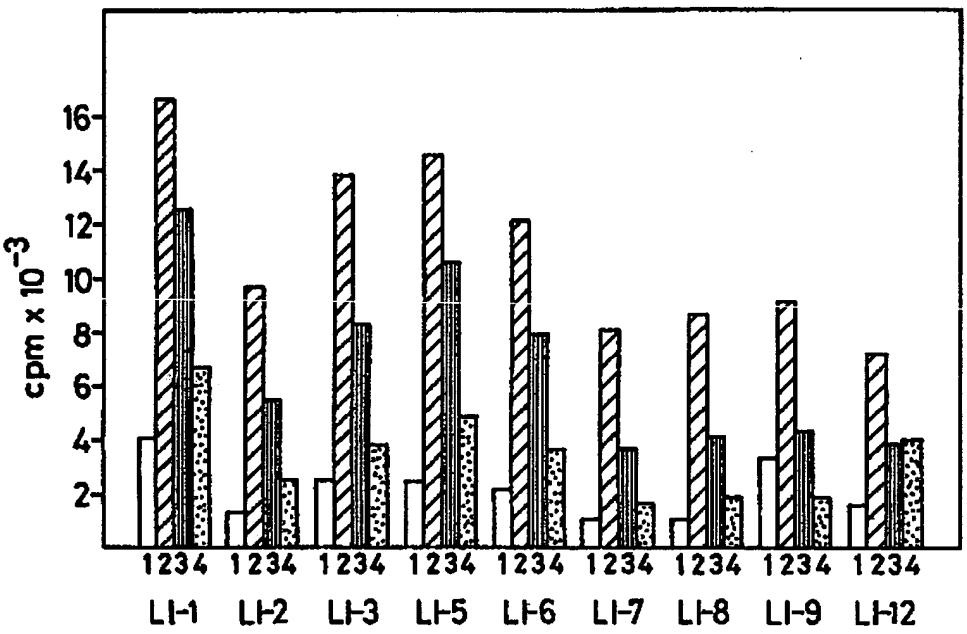
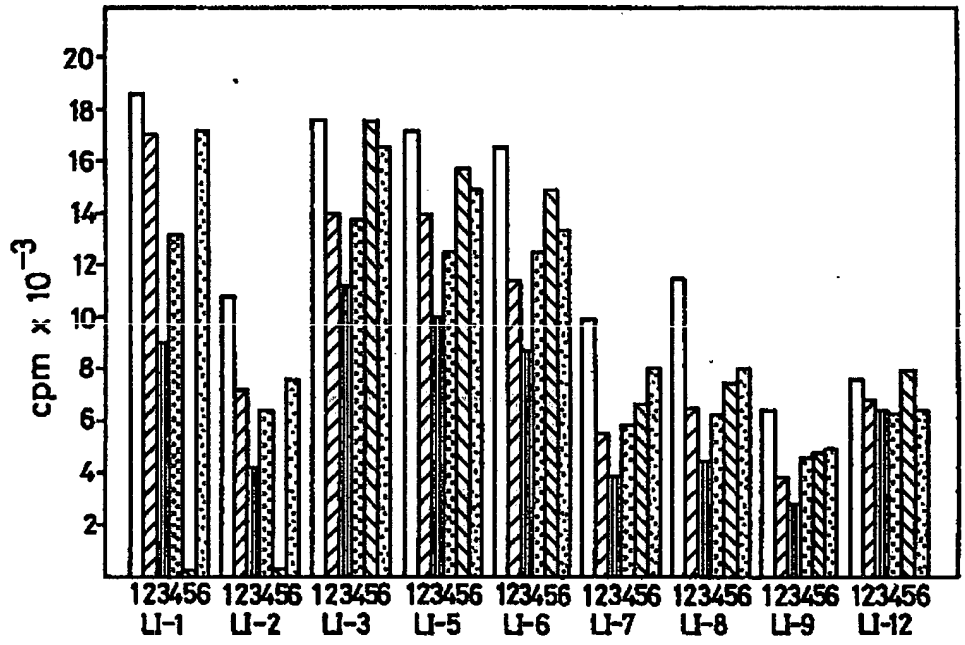


1 = IFL α
2 = IFL β
3 = IFL γ
4 = IFL δ

cpm = specific binding per test (after deduction of 300-400 cpm of non-specific binding against BSA; LI-12 non-specific binding about 1200 cpm)

FIG. 1



1 = IFL α_1

2 = IFL α_2

3 = IFL β_2

4 = IFL γ_2

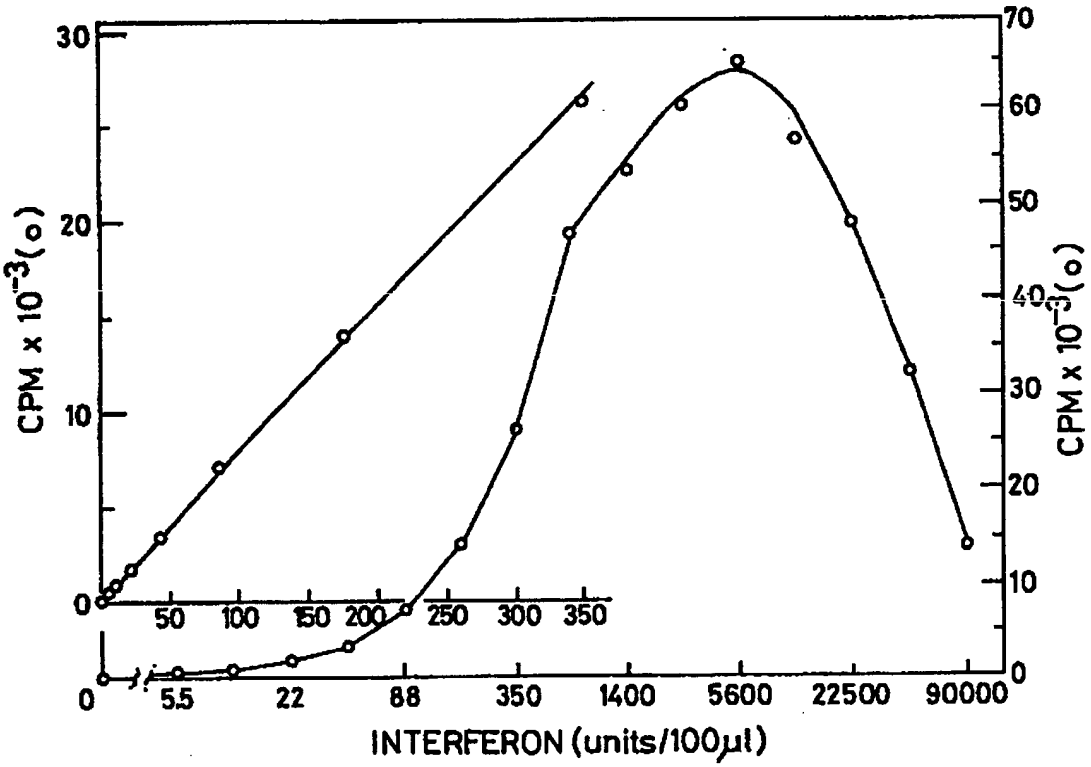
5 = IFL γ_3

6 = IFL K

cpm = specific binding per test (after deduction of 300-400 cpm of non-specific binding against BSA; LI-12 non-specific binding about 1200 cpm)

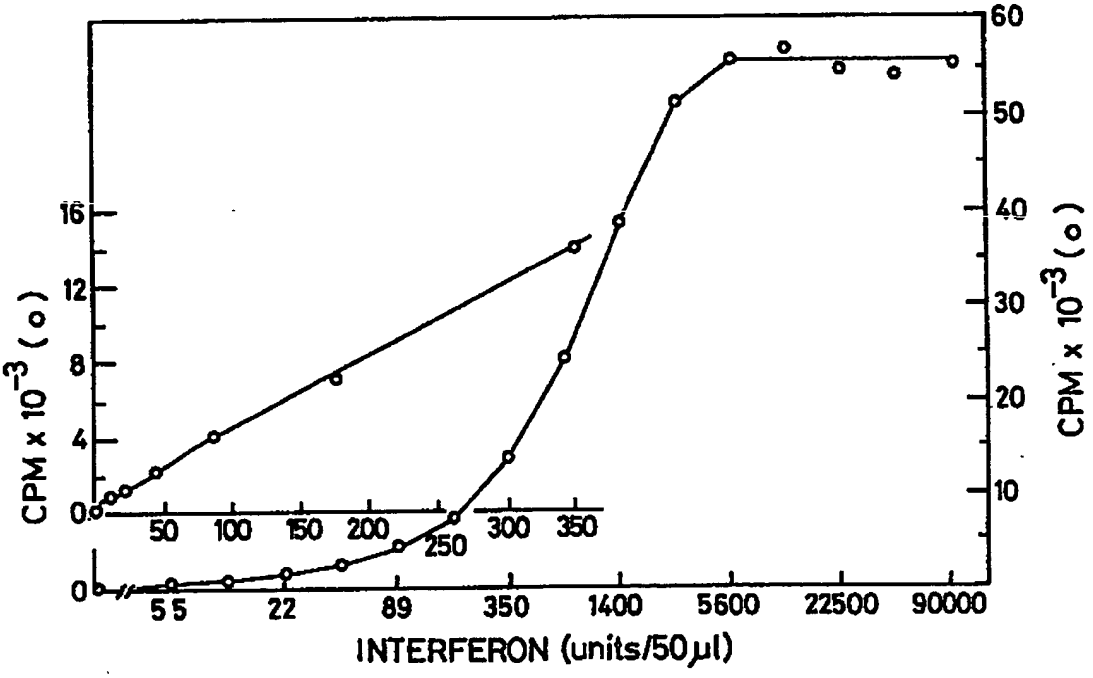
FIG. 2

FIG. 3



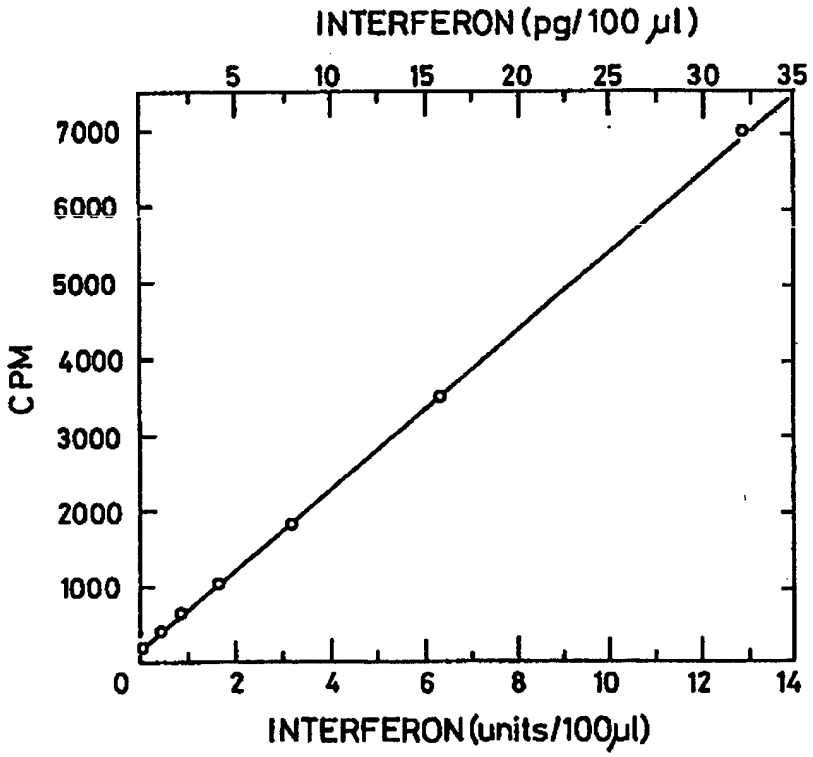
cpm: ^{125}I-LI-1 bound per test; interferon concentration (abscissa) is plotted logarithmic for the main curve (•—•) and plotted linear for the curve (•—•) inserted on the left.

FIG. 4



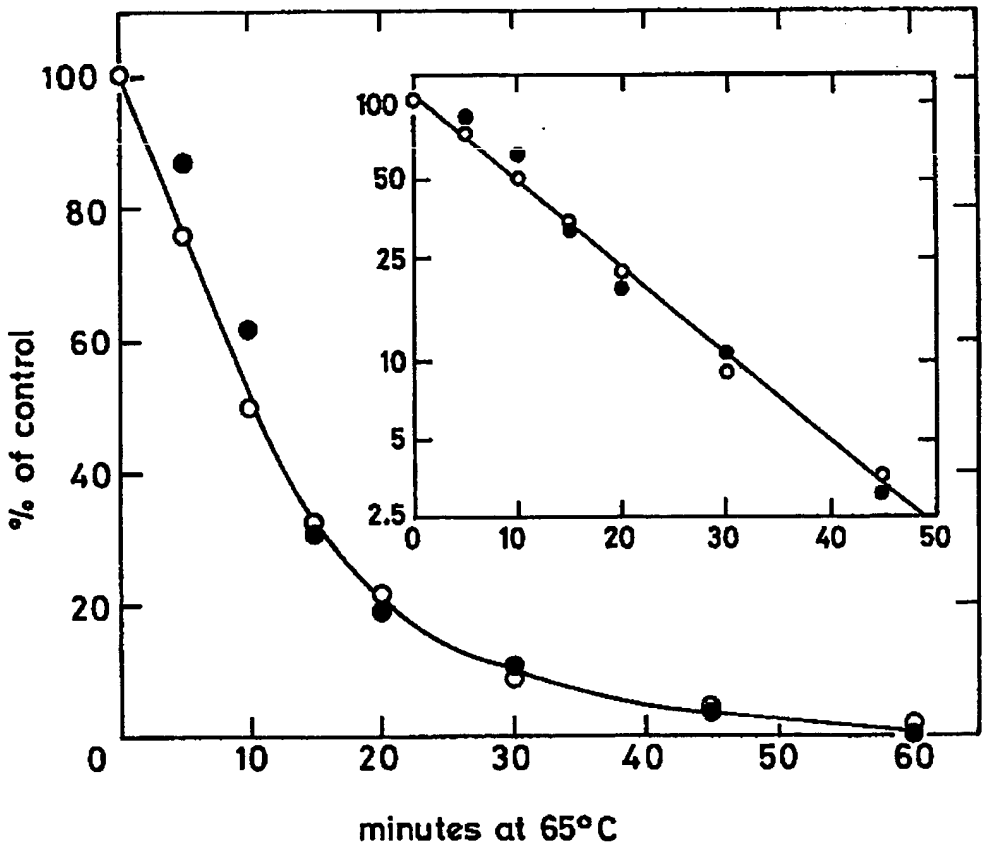
cpm: ^{125}I-LI-1 bound per test; interferon concentration (abscissa) is plotted logarithmic for the main curve (•——•) and plotted linear for the curve (•——•) inserted on the left.

FIG. 5



cpm: see FIG. 3, inserted curve.

FIG. 6



legend ○ = radio immuno assay

● = CPE inhibition test

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